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Seroprevalence Against Norwalk-Like Human Caliciviruses in Beijing, China

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Serum specimens from 1,109 individuals at different ages in Beijing, China, were collected between April 1996 and March 1997 and tested for IgG antibodies against human caliciviruses using enzyme immune assays (EIAs). Baculovirus-expressed recombinant Norwalk virus (rNV) and Mexican virus (rMxV) capsid proteins were used as antigens. The seroprevalence was 89% for rNV and 91% for rMxV. Similar seroprevalence between the two antigens was observed in individual age groups and both genders. Infants had a high seroprevalence (99% for NV and 94% for MxV) at birth. The lowest seroprevalence (41% for rNV and 36% for rMxV) was at 7–11 months of age. A sharp increase in seroprevalence occurred in early childhood, with 65% and 70% at one, 85% and 90% at three, and 100% and 98% at 8–9 years of age for rNV and rMxV, respectively. Forty-three individuals had antibody against rNV but not rMxV and 63 individuals had antibody against rMxV but not rNV, indicating different levels of exposure to the two strains in these individuals. This is the first report of surveillance of antibodies against NV-like viruses in China. The observed high prevalence and early age antibody acquisition suggest that infection by these two human calicivirus strains is common in this population. ***J. Med. Virol. 60:97–101, 2000.*** © 2000 Wiley-Liss, Inc.

KEY WORDS: Norwalk-like viruses; human caliciviruses; seroepidemiology; recombinant EIAs

INTRODUCTION

Norwalk virus (NV)-like viruses are important viral pathogens causing acute gastroenteritis in humans. Early studies estimated that 30–50% of non-bacterial gastroenteritis and 90% of food-borne outbreaks of gastroenteritis were associated with NV-like viruses [Kapikian et al., 1996]. Such outbreaks usually have high attack rates and occur in schools, restaurants, summer camps, hospitals, nursing homes, and cruise ships. Exposure to a common source of viruses, such as contaminated food or water, can usually be identified. Outbreaks resulting from consumption of uncooked shellfish are common. NV-like viruses also can be spread by person-to-person transmission.

Under electron microscopy, NV-like viruses are small, round, and 27–35 nm in diameter and the viruses reveal surface structures that are distinct from morphologically typical caliciviruses [CVs, Kapikian et al., 1972]. Therefore, NV-like viruses are also called small round structured viruses (SRSVs). The cloning and sequencing of many prototypes and new isolates of NV-like viruses confirmed the classification of NV-like viruses as human CVs (HuCVs), in addition to the morphologically typical CVs [Jiang et al., 1990, 1993, 1995b, 1997; Lambden et al., 1993; Liu et al., 1995]. The NV-like HuCVs are divided into two genogroups, one genogroup containing the prototype NV and the other genogroup containing the prototype Snow Mountain virus [SMV, Jiang et al., 1996; Berke et al., 1997]. Genetic characterization of NV-like HuCVs also allowed development of new diagnostic assays, including enzyme immune assays (EIAs) based on reagents generated from baculovirus-expressed viral capsid antigens and reverse transcription-polymerase chain reaction (RT-PCR), for detection of viral RNA in stool specimens [Jiang et al., 1992, 1995a, 1996; Graham et al., 1994; Parker et al., 1993, 1995; Treanor et al., 1993].

Acute gastroenteritis is one of the most important infectious diseases in China as in many developing countries; however, there is only one report of HuCV

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studies in China [Fang et al., 1995]. Large-scale studies of HuCVs-associated gastroenteritis have not occurred. In this study, EIAs were used based on baculovirus-expressed recombinant HuCV capsid antigens to describe seroprevalence of IgG antibodies against HuCVs in Beijing, China, the capital and second-largest city of the country. Two baculovirus-expressed capsid antigens, recombinant NV (rNV) and recombinant Mexico virus (rMxV), were used. The NV belongs to the NV-genogroup and the MxV belongs to the SMV genogroup [Jiang et al., 1996; Berke et al., 1997]. Antigenically, NV and MxV are distinct but share minor epitopes [Jiang et al., 1996]. Therefore, this seroprevalence study represents results for two genogroups that may contain at least two antigenic types.

MATERIALS AND METHODS

Serum Specimens

Blood specimens were collected from children and adults undergoing regular physical examination at the affiliated Childrens Hospital of the Capital Institute of Pediatrics and at Xuanwu Hospital in Beijing, between April 1996 and March 1997; 1,109 blood specimens were collected from individuals whose age was from birth to over 60 years. Sera were kept at –20 °C before testing.

rNV and rMxV Capsid Antigens

The baculovirus-expressed rNV and rMxV capsid antigens were derived from insect cells infected with recombinant baculoviruses carrying the NV and MxV capsid genes, respectively. The MxV was originally isolated from a child with acute gastroenteritis in Mexico in 1990 [Jiang et al., 1995b]. The expressed capsid antigens were purified by a sucrose gradient followed by a CsCl gradient centrifugation, as described previously [Jiang et al., 1992, 1995a].

Enzyme Immune Assays (EIAs) to Detect Antibodies Against NV and MxV

The original EIAs to detect antibodies against NV and MxV [Jiang et al., 1992, 1995a] were employed. Purified rNV and rMxV capsid proteins, at a concentration of 1 μg/ml in 0.01 M phosphate buffered saline (PBS, pH 7.4), were used to coat 96-well microtiter plate at 50 μl per well. The plate was incubated overnight at 4°C. The antigen-coated microtiter plate was washed once (200 μl per well) with 0.05% Tween 20-PBS (TW-PBS) and then blocked with 5% Blotto (Carnation nonfat milk) in PBS for 1 hour at 37°C. After washing twice (200 μl per well) with TW-PBS, 50 μl of human serum specimens at 1:200 dilution in 1% Blotto-PBS were added to the wells and the plate was incubated for 2 hours at 37°C. After six washes with TW-PBS, 50 μl of horseradish peroxidase-conjugated goat anti-human immunoglobulin G (IgG, GIBCO BRL, Gaithersburg, MD) at 1:8,000 dilution in 1% Blotto-PBS was added to each well and the plate was incubated for 2 hours at 37°C. After a final six washes with TW-PBS, the bound antibody was detected by addition of 3,3,5,5-tetramethylbenzidine (TMB, GIBCO BRL, Gaithersburg, MD). After a 10 min incubation at room temperature, a solution of 1.0 N H_2SO_4 was added to stop color development. The reactivity of each well was quantified at optical density 450 (OD_{450}) by an Emax microtiter plate reader (Molecular Devices, Sunnyvale, CA).

All incubation steps, including coating, blocking, and antibody reaction, were performed in humid chambers. The optimal concentrations of the coating antigens and the second antibody for the assays were determined by titration. Positive and negative serum controls, as well as rNV and rMxV antigen controls, second antibody controls, and substrate controls, were included in each test. A specimen with an $OD_{450} \geq 0.2$ was considered to be positive. This cut-off point was verified by a comparison with a group of negative control serum specimens.

Statistical Analysis

χ^2 analysis was performed to compare the antibody detection rates between the two recombinant EIAs in different age and gender groups.

RESULTS

Age-Specific Serum IgG Antibodies Against rNV and rMxV

As shown in Table 1, among the 1,109 serum specimens tested, the seroprevalence of IgG antibodies against rNV and rMxV was 89% and 91%, respectively. The seroprevalence for the two antigens was similar within each age group and between genders. Seroprevalence was 99% for NV and 94% for MxV at birth (< 2 months). A rapid decrease occurred after birth, with the lowest levels (41% for rNV and 36% for rMxV) at 7–11 months of age. A sharp increase of seroprevalence occurred in early childhood, reaching 65% and 70% for rNV and rMxV, respectively, at 1 year of age, 85% and 90 % at 3 years of age, and 100% and 98% at 8–9 years of age, when most children started elementary school. The seroprevalence remained nearly 100% for older age groups.

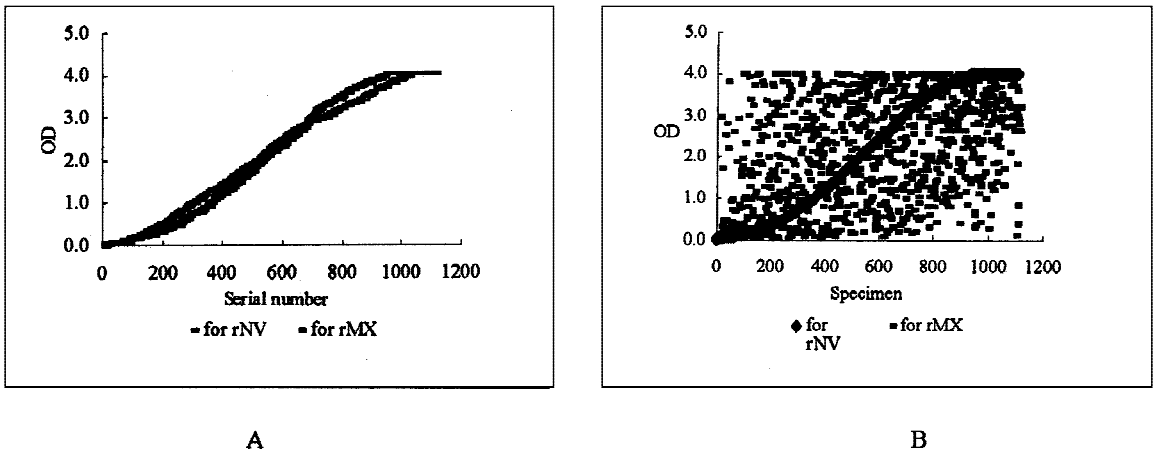
Age-Specific OD Values for rNV and rMxV EIAs

Because the OD values of an EIA are expected to reflect antibody concentration, we compared the OD values of the two EIAs to estimate the past exposure to the two strains. When the rNV and rMxV OD values of the 1,109 individuals were plotted separately, similar curves were observed for the two recombinant antigens (Fig. 1A). However, the OD values for rMxV were widely scattered when plotted against the OD values for rNV (Fig. 1B). Forty-three (4%) individuals had antibody against rNV but not rMxV, and 63 (6%) individuals had antibody against rMxV but not rNV, indicating these individuals had different exposures to the two strains in the past. The OD values by the two EIAs correlate with the antibody detection rates among different age groups (Fig. 2). However, slight differences of OD values between the two antigens were found in some age groups. For example, an increase of OD val-

TABLE I. Detection of Serum IgG Antibodies Against Norwalk-Like HuCVs Using the Baculovirus-Expressed Recombinant Norwalk Virus (rNV) and Mexico Virus (rMxV) Capsid Antigens in a Population of Beijing, China

	Number samples		Number (%) rNV-positive		Number (%) rMxV-positive	
Age	Total	M/F[b]	Total	M/F	Total	M/F
<2 months	79	34/45	78 (99)	34 (100)/44 (98)	74 (94)	32 (94)/42 (93)
2–3 months	39	28/11	32 (82)	25 (89)/7 (64)	36 (92)	26 (93)/10 (91)
4–6 months	68	42/26	46 (68)	27 (64)/19 (73)	47 (69)	30 (71)/17 (65)
7–11 months	58	35/23	24 (41)	19 (54)/5 (22)	21 (36)	16 (46)/5 (22)
1 year	46	26/20	30 (65)[a]	17 (65)/13 (65)	32 (70)[a]	17 (65)/15 (75)
2 years	44	22/22	34 (77)	18 (82)/16 (73)	34 (77)	17 (77)/17 (77)
3 years	39	17/22	33 (85)	17 (100)/16 (73)	35 (90)	17 (100)/18 (82)
4 years	40	18/22	34 (85)	16 (89)/18 (82)	37 (93)	16 (89)/21 (96)
5 years	32	16/16	27 (84)	12 (75)/15 (94)	30 (94)	15 (94)/15 (94)
6–7 years	61	31/30	50 (82)	26 (84)/24 (80)	58 (95)	31 (100)/27 (90)
8–9 years	62	31/31	62 (100)	31 (100)/31 (100)	61 (98)	30 (97)/31 (100)
10–11 years	62	31/31	62 (100)	31 (100)/31 (100)	62 (100)	31 (100)/31 (100)
12–13 years	60	32/28	56 (93)	30 (94)/26 (93)	60 (100)	32 (100)/28 (100)
14–15 years	55	30/25	55 (100)	30 (100)/25 (100)	55 (100)	30 (100)/25 (100)
16–19 years	57	28/29	56 (98)	27 (96)/29 (100)	57 (100)	28 (100)/29 (100)
20–29 years	61	31/30	60 (98)	30 (97)/30 (100)	59 (97)	29 (94)/30 (100)
30–39 years	62	33/29	62 (100)	33 (100)/29 (100)	62 (100)	33 (100)/29 (100)
40–49 years	64	32/32	64 (100)	32 (100)/32 (100)	63 (98)	31 (97)/32 (100)
50–59 years	63	32/31	63 (100)	32 (100)/31 (100)	63 (100)	32 (100)/31 (100)
>60 years	57	28/29	57 (100)	28 (100)/29 (100)	57 (100)	28 (100)/29 (100)
Total	1,109	577/532	985 (89)	513 (89)/472 (89)	1005 (91)	521 (90)/484 (100)

[a]A significant increase of antibody detection rate compared with the next younger group (rNV, $P < 0.05$; rMxV, $P < 0.01$).
[b]M, male; F, female.



A B

Fig. 1. Distribution of the EIA OD values of serum specimens collected from Beijing residents. The OD values of the 1,109 serum specimens for the rNV (ODnv) and rMxV (ODmx) EIAs were plotted independently for each antigen used (**A**), or together according to an order of increased ODnv values (**B**).

ues (all three curves) occurred after 7–11 month of age for both antigens, but the increase for rMxV was sharper (Fig. 2).

DISCUSSION

EIAs based on baculovirus-expressed recombinant capsid antigens have been applied for epidemiological studies of NV-like HuCVs in several countries [Gray et al., 1993; Lew et al., 1994; Parker et al., 1994; Dimitrov et al., 1997; Wolfaardt et al, 1997; Smit et al., 1997]. In this study, two such recombinant capsid antigens, representing two major HuCV genogroups were used simultaneously in seroprevalence study. The high seroprevalence against rNV and rMxV (about 90%) in Beijing residents suggests both genogroups co-circulate in the city. Because NV and MxV share some antigenic epitope(s), which also could be shared with other strains in these genogroups, NV and MxV may be markers for exposure to strains of these genogroups not genetically identical with NV and MxV. The high levels of maternal antibodies found in newborns indicate a high exposure to the strains in adult women. The sharp increase in seroprevalence in early childhood suggests a high frequency of primary infection in young children. Most children at this age in large cities of China are sent to child care centers, kindergarten, and elementary schools, where increased exposure is likely to occur. The high seroprevalence against the two strains in adult age groups suggests that either a repeated exposure to the two strains occurred or the antibodies

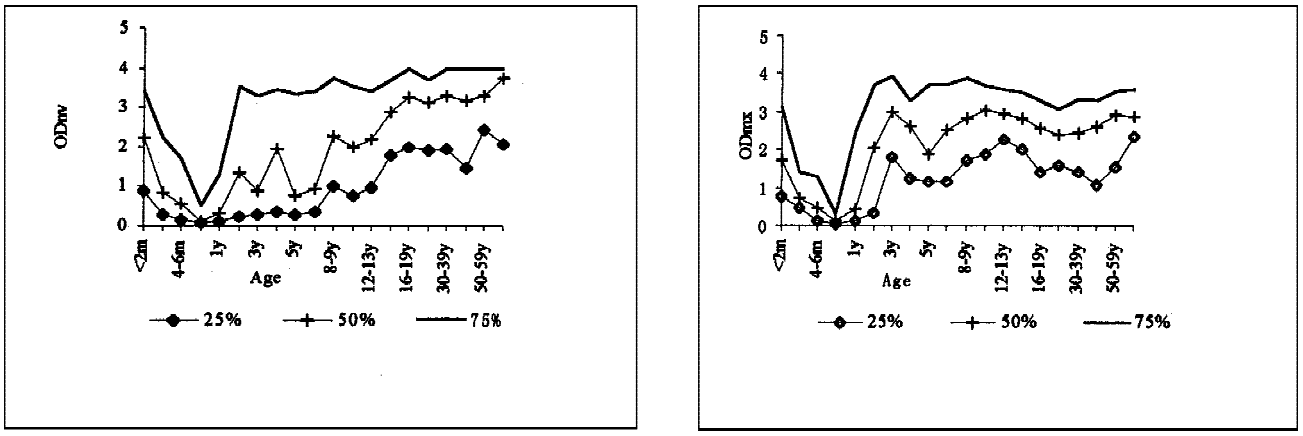


Fig. 2. Distribution of age specific OD values of serum specimens collected from Beijing residents. The OD values of EIAs reached by 25%, 50%, and 75% of individuals in each age group were plotted. **A:** For rNV; **B:** for rMxV. All the 1,109 serum specimens were included in both panels.

persisted following primary infection. The latter is unlikely because earlier studies suggested that the immunity to NV infection is short-lived [Johnson et al., 1990].

High prevalence of antibodies against two antigenic types of HuCVs in one geographical location has been found in other countries. For example, in Mexico and Kuwait, children had high seroprevalence against NV and MxV at early ages and seroprevalence remained high at older ages [Jiang et al., 1995b; Dimitrov et al., 1997]. Lower seroprevalence against NV was reported in the United States in early studies [Greenberg et al., 1979a,b; Kapikian et al., 1996]. This could be due to a low sensitivity of tests used in those studies. Using baculovirus-expressed viral capsid antigens, high seroprevalence against HuCVs was reported in many developed countries, including England [Gray et al., 1993; Parker et al., 1994], Japan [Numata et al., 1994], and Finland [Lew et al., 1994], and in both developed and underdeveloped areas in Africa [Wolfaardt et al., 1997; Smit et al., 1997].

NV-like HuCVs usually cause large outbreaks of acute gastroenteritis in developed countries. In developing countries, large outbreaks also occur, but sporadic infections are more common. Poor living conditions may result in high exposure (frequency and inoculum) to a pathogen, which could result in early herd immunity preventing the large outbreaks in adults common in developed countries. The transmission pattern of NV-like HuCVs in Beijing remains to be determined. Consumption of raw seafood is not popular in Beijing. Sanitation conditions for Beijing residents have improved significantly in the past 10 years; however, overcrowded housing conditions remain. Therefore, person-to-person transmission may play a major role in infection. Large outbreaks of acute gastroenteritis have been reported in Beijing [Qian et al., 1994]. Whether these outbreaks are caused by HuCVs remains unknown. Active surveillance is necessary. With the recent development of the recombinant EIAs and RT-PCR techniques, such surveillance now is possible.

Because it is difficult to test all serum specimens by titration, we analyzed the OD values of individual specimens in an attempt to estimate the levels of antibody to the two strains. When the OD values to the two antigens in the population were compared, wide variations were observed. Among the 1,109 individuals studied, 106 (10%) had antibodies to one antigen but not to the other, suggesting different exposures to two strains in the past. Different OD values also were found among different age groups. For example, OD values increased for both antigens in early childhood, but MxV OD values increased more sharply than NV OD values before 3 years of age, indicating the MxV infection might be more common than NV infection in this age group. Several studies have suggested that strains in the NV genogroup were predominant in the 1970s but strains in the SMV genogroup have become more common currently [Jiang et al., 1996]. It was noticed that both OD value and prevalence of maternal antibody to rNV was higher than to rMxV in children of Beijing. This indicates that NV circulated more commonly than MxV in the past in this area. A preliminary conclusion is that the prevalence of different HuCVs follows a dynamic pattern in Beijing. Future studies to test this hypothesis are necessary.

In summary, the epidemiological pattern of NV-like HuCVs in Beijing area has the following features: at least two genogroups and two antigenic types of HuCVs co-circulate, children acquire infection at early ages, high infection rates occur in both genders, all age groups are exposed to strains in the two genogroups, and person-to-person contact may be a major route of transmission.

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